

Inductively-Coupled RF Powered O₂ plasma as a Sterilization Source

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Low-temperature or cold plasmas have been shown to be effective for the sterilization of sensitive medical devices and electronic equipment¹. Low-temperature plasma sterilization procedures possess certain advantages over other protocols such as ethylene oxide, gamma radiation and heat^{2,3} due to the use of inexpensive reagents, the insignificant environmental impacts and the low energy requirements. In addition, plasmas may also be more efficacious in the removal of robust microorganisms due to their higher chemical reactivity. Together, these attributes render cold plasma sterilization as ideal for the surface decontamination requirements for NASA Planetary Protection. Hence, the work described in this study involves the construction, characterization and application of an inductively-coupled, RF powered oxygen (O₂) plasma.

Current theories regarding the physical basis of oxygen plasma sterilization are based on the effects of reactive oxygen species and ultraviolet radiation^{4,5}. The biochemistry resulting from the plasma-surface interactions, however, is not well characterized. Thus, the present research is designed to provide insights into the interaction of low-temperature plasmas with biological matter. Our experimental strategy involves (1) characterization of the plasma composition using spectroscopic methods and (2) biochemical and microbiological analysis of the plasma reactivity. The plasma composition was experimentally characterized in two companion facilities and its reactivity assayed by measuring cell survival and DNA fragmentation using a model microbe and biomolecule, respectively.

The compositional characterization of the O₂ plasma was performed in a gaseous electronics conference (GEC) cell. A detailed description of the facility is provided by Kim *et al.*,⁶ and is briefly reviewed here. The GEC cell is a high vacuum inductively coupled reactor in which an O₂ plasma is produced by applying 13.56 MHz RF power to a flat 5-turn 89 mm diameter inductive coil made of 3.175 mm copper tube. The coil is insulated from the plasma by a quartz window. A 100 mm diameter stainless steel disk, which serves as the lower-electrode, is integrated both electrically and mechanically to an electrostatic quadrupole plasma analyzer (EQPA). The EQPA chamber is differentially pumped to a base pressure of 2×10^{-9} Torr by a turbo molecular pump backed by a mechanical pump. The high transmission EQPA (Hiden Analytical Ltd.) – consisting of a combination of ion transporting lenses, 45° sector-field ion energy analyzer, quadrupole mass filter (QMF), and ion detector (channeltron) – is used to measure the ion energy distribution (IED), ion flux and mean energy of the ions produced in the plasma. A 10 microns hole in the center of the lower-electrode serves as the sampling orifice for the EQPA. A commercially available RF compensated Langmuir probe made by Scientific Systems, Inc. has been used to measure the plasma parameters in this study. The body of the probe is made of a cylindrical and hollow ceramic tube with a tapered ceramic tip holder. A platinum wire, 380 μ m in diameter and 5 mm in length, is used as the probe tip. The probe is used to measure the electron and ion number densities, plasma potential, electron energy distribution function and electron temperature.

Emission spectra are obtained by using Thermovision Colorado model 82-050 Spectrograph with a 1200 lines/mm grating and a Princeton Instrument linear diode array with a dispersion of 2.5 pixels/Å. This combination of collection optics is capable of providing a wide spectral range of emission data (2000 to 9000 Å).

Preliminary Results

GEC Cell:

The first set of experiments were conducted at 50 mTorr of pressure and 200 W of input power. Based on Langmuir probe measurements the electron number density is found to be $7 \times 10^9 \text{ cm}^{-3}$ and positive ion number density to be $4.5 \times 10^{10} \text{ cm}^{-3}$. The electron temperature is measured to be 7.2 eV and the plasma potential to be 18.8 V. The corresponding electron energy probability function (EEPF) shown in Fig. 1 suggests a non-Maxwellian plasma, which may be approximated by a two-temperature model representing high and low energy electrons. O_2^+ appears to be the dominant ion as seen from the mass scan from the EQPA shown in Fig. 2 with the mean ion energy of 16 V (see inset in Fig. 2). The emission data are shown in Fig. 3. Strong UV molecular bands (O_2^+ and O_2) dominate in the 2500-3200 Å spectral range. Atomic O lines dominate the visible and IR regions.

Biochemical Assay:

As a model system, plasmid DNA was exposed to the oxygen plasma (500 mtorr, 200 W) for differing times and analyzed by agarose electrophoresis. Several 1 µg samples of plasmid DNA (pRc/CMV2, Sigma Co.) were dried onto hanging drop microscope slides and exposed to the oxygen plasma for 0, 30, 60 and 180 seconds. The samples were redissolved in 25 mM HEPES (pH 8.0), run on a 1% agarose gel and visualized using ethidium bromide. The effects of the oxygen plasma on the structural integrity of DNA are displayed in Figure 4. The results indicate that the DNA is rapidly degraded by double-stranded fragmentation (as observed by broad low molecular weight bands in lanes 3 and 4), crosslinking (as seen in lane 4) and volatilization (inferred by loss of sample in lane 5).

Microbiological Assay:

Deinococcus radiodurans was chosen as a model microbe due its high radiation resistance. Approximately 4×10^6 cells were transferred onto a 25 mm Nucleopore membrane and dried. The inoculated membrane was exposed to the oxygen plasma (500 mtorr, 100 W) for 5 seconds, removed and placed into 0.5 mL minimal media. Serial dilutions of the media were plated on TGY/agar plates and incubated for 3 days at 30 °C. Colonies were counted and compared to control samples. Results indicated that < 5% of the cells survived after the 5 sec exposure.

Conclusion

Oxygen plasmas contain several reactive species such as atomic oxygen and dioxygen cations. These species are responsible for the efficient sterilization of contaminated samples (95% removal in 5 sec). The oxygen plasma can rapidly degrade DNA, which may be an important mechanism of sterilization. In all, this research will

provide a comprehensive study of the emission spectra, ion kinetics and plasma parameters at the various operating conditions necessary for effective sterilization.

References

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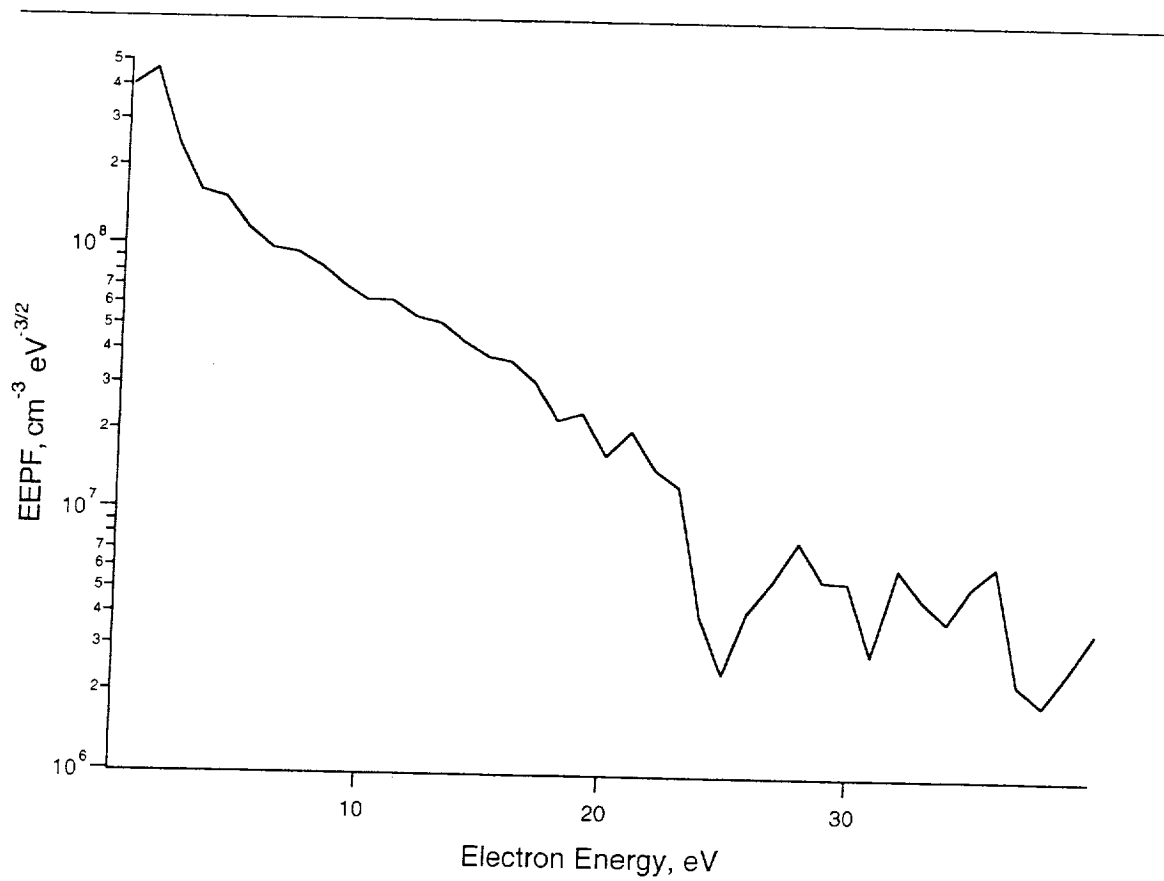


Figure 1. EEPF at 50 mtorr and 200 W.

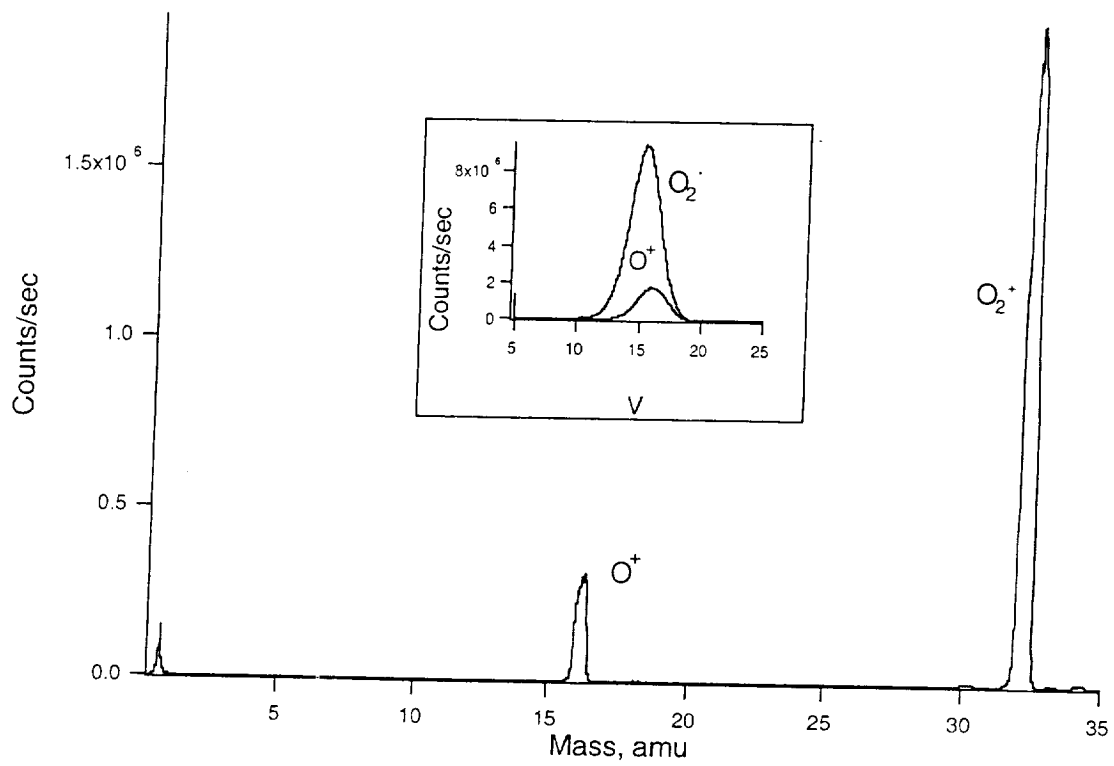


Figure 2. Mass spectrum (50 mTorr, 200 W)

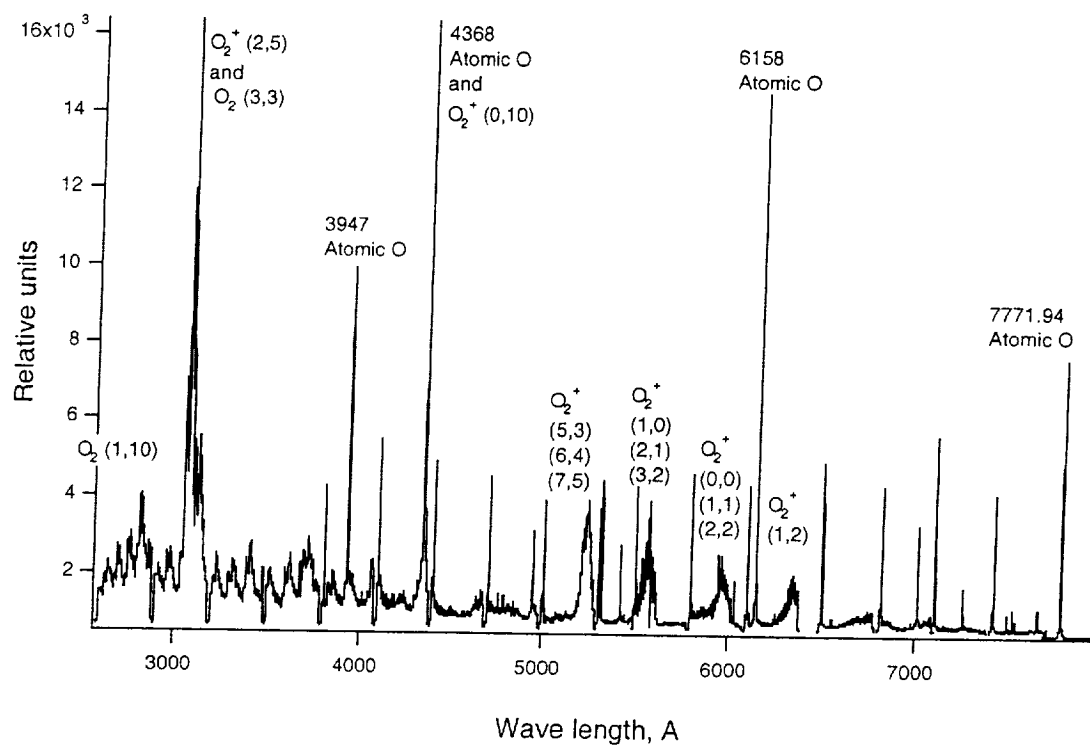


Figure 3. Emission spectrum.

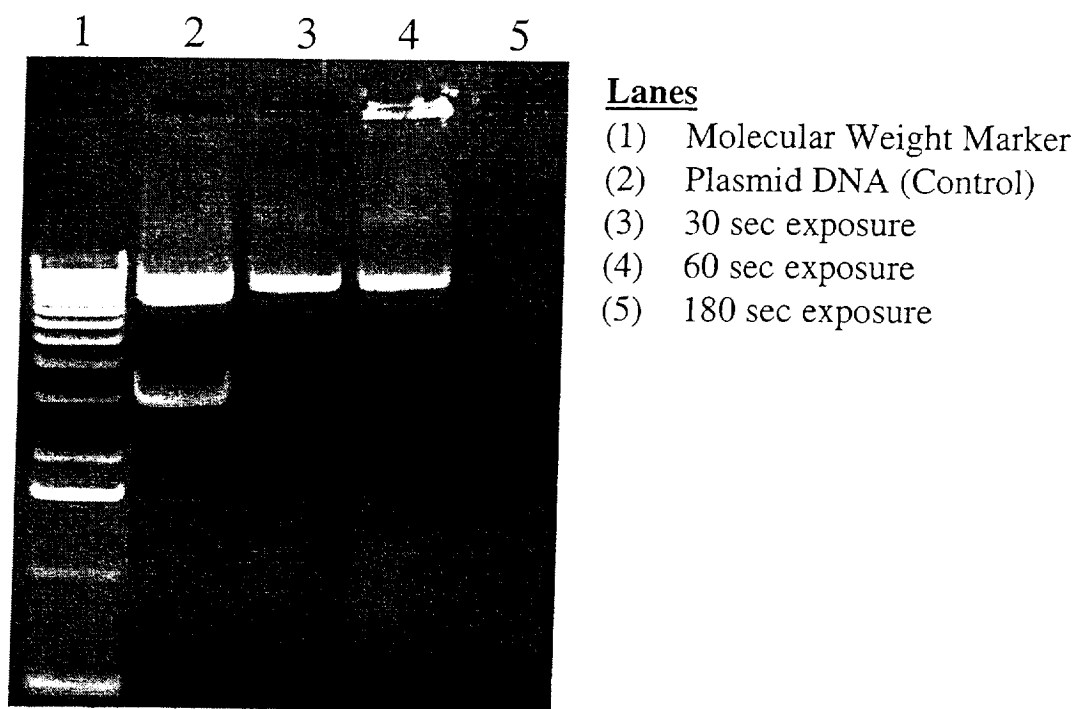


Figure 4. DNA Degradation.

